JEE Journal of Ecological Engineering

Journal of Ecological Engineering, 2025, 26(10), 272–290
https://doi.org/10.12911/22998993/206961
ISSN 2299–8993, License CC-BY 4.0

Received: 2025.05.29
Accepted: 2025.07.15
Published: 2025.07.22

Study of the effect of bacterial bioactivator on the content of phenolic compounds in *Salvia sclarea* L.

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ABSTRACT

The aim of this work is to study the content of biologically active substances in medicinal plants when treated with bacterial fertilizer. As part of the experiment, studies were conducted on growing *Salvia sclarea* L. using environmentally friendly biofertilizers (bacterial fertilizer TERIA-S). The use of these fertilizers stimulates the development of both the root system and the above-ground part of the plant. Standard methods of soil microbiology were used to analyze the agrochemical and microbiological composition of the soil in which the plants were cultivated. Cultivation of *Salvia sclarea* L. using TERIA-S was first carried out in the laboratory and then in the field. After harvesting the *Salvia sclarea* L., the content of phenolic compounds was studied using the Folin-Ciocalteu method. Then extraction and analysis were carried out using high-performance liquid chromatography (HPLC). The antimicrobial activity of phenolic compounds was studied using the well-diffusion agar method. The plants treated with the biopreparation exhibited superior performance across all phenological phases compared to the control group. Notably, the root system of the treated plants was 1.5 times longer and more robust, indicating a significant enhancement in growth and vigor. HPLC analyses revealed the presence of several bioactive compounds within the *Salvia sclarea* L. samples. Specifically, kaempferol was detected in all samples, while quercetin was identified in the butanol and ethyl acetate fractions. Additionally, apigenin and luteolin-7-glycoside were present in the butanol fraction, suggesting that these compounds may contribute to the plant's bioactivity. The ethanol fractions of *Salvia sclarea* L. exhibited considerable antioxidant activity, with a recorded inhibition of 79.80 ± 0.14%. Furthermore, these fractions demonstrated antimicrobial efficacy against *Bacillus subtilis* and *Candida albicans*, with inhibition zones measuring 12 mm for both microorganisms. These findings highlight the potential of *Salvia sclarea* L., particularly its ethanol extracts, as a source of bioactive compounds with both antioxidant and antimicrobial properties. The observed enhancements in plant growth and secondary metabolite production suggest that the biopreparation not only supports plant development but also may contribute to the increased production of compounds with pharmacological relevance.

Keywords: bioactivator, medicinal plants, extraction, bioactive compounds, flavonoids.

INTRODUCTION

Since ancient times, medicinal plants have been utilized across various cultures for the treatment of a wide range of diseases. Scientifically, the therapeutic efficacy of these plants is primarily attributed to the biologically active compounds they contain, which exert significant influence on biological processes in both animals and humans (Davis and Choisy, 2024). Among these bioactive constituents, phenolic compounds, which are secondary metabolites in plants, play a pivotal role (Zang et al., 2022). Phenolic compounds are characterized by the presence of aromatic rings bearing hydroxyl groups, along with a diverse array of derivatives (Al Mamari, 2022). The diversity of phenolic compounds can be attributed to the variation in the carbon skeletons and the numerous positions at which substitutions can occur on the benzene ring, leading to the identification of over 8,000 distinct phenolic structures to date (Alara et al., 2021). This remarkable structural diversity underpins the wide-ranging applications of these compounds in fields such as medicine (Saibel et al., 2021), the food industry (Martillanes et al., 2017), and the development of advanced nanomaterials (Lawal et al., 2025). However, the most significant application of phenolic compounds lies within pharmacology. Their well-documented ability to exhibit a range of pharmacological activities – including antioxidant, anti-inflammatory, antimicrobial, antispasmodic, neuroprotective, antibacterial, anticoagulant, and immunostimulatory effects – has facilitated their use in the development of pharmaceuticals that are less toxic than their synthetic counterparts (Hemaiswarya et al., 2008).

The diversity of phenolic compounds, with their various structural configurations, is intricately linked to the performance of a wide array of functions within the plant organism. These compounds play a crucial role in several fundamental physiological processes, including photosynthesis, respiration, growth, and adaptation to environmental conditions. Furthermore, they are involved in the suppression of seed germination, among other functions. Phenolic compounds are integral to the plant's defensive mechanisms, playing a key role in protective responses to biotic and abiotic stressors. In the event of pathogen attacks or under stressful environmental conditions, the concentration of these compounds can increase substantially. In the work of Olechenko, for example, cold hardening of wheat plants showed an increase in the content of phenolic substances by 2.5–3 times (Olenichenko et al., 2006). Also, phenolic compounds protect plants from a number of unfavorable environmental factors, including ultraviolet radiation, temperature stress, and increased concentrations of substances (Mierziak et al., 2014). Numerous data have been accumulated on the effect of these compounds on other protein and non-protein structures, which can lead to a change in the functional state of cells and the entire plant organism as a whole (Joyner, 2021). They can also regulate the functions of vital cellular enzymes in the animal organism and are potent inhibitors of various enzymes (Walker et al., 2000).

Due to the rich variety of biologically active substances in their composition, medicinal plants have become objects of study by many scientists in recent years. One of the widely used medicinal plants is clary sage (*Salvia sclarea* L.). It is an aromatic plant belonging to the Lamiaceae family. Clary sage is known for its valuable essential oil, which is widely used in the perfume industry due to its persistent and refreshing aroma. In addition, the oil has various medicinal properties and is used for stress, tension, depression, insomnia, etc. (Yaseen et al., 2014). Traditional uses of clary sage oil include its use against gingivitis and stomatitis, while recent studies have reported analgesic, anti-inflammatory, antimicrobial, and cytotoxic effects (Gulcin et al., 2004), and the drug is well known as a natural source of antioxidants. The antioxidant properties of sage plants, as well as their high and diverse biological activity, are usually explained by the phenolic compounds in their composition. However, it should be taken into account that the chemical composition and biological activity of plant extracts are strongly influenced by abiotic factors (Durling et al., 2007). Eighteen phenolic compounds identified using international standards have been found in *S. sclarea* extracts. They consist of six flavonoids and twelve phenolic acids (Jasicka-Misiak et al., 2018).

The primary indicator of the quality of plant materials intended for pharmaceutical applications is the concentration of biologically active compounds. The chemical composition of plants and the concentration of these active components can exhibit considerable variability, influenced by a range of factors. Notably, the same plant species may demonstrate significant differences in chemical composition depending on the geographic region in which it grows. For example,

in a study conducted by Stanislav Sukhikh et al., which examined the chemical composition and content of biologically active substances in *C. coggygria*, *D. maculata*, and *P. chlorantha* growing in the Eastern Baltic States, it was found that plants from this region contained a higher concentration of biologically active substances, vitamins, and mineral elements compared to specimens from other regions. This variation was primarily attributed to the distinct climatic conditions of the Eastern Baltic (Sukhikh et al., 2021). Similarly, research by Wei Liu et al. on *P. fruticosa* growing in various regions of China demonstrated that the annual duration of sunlight and altitude above sea level positively correlated with higher concentrations of rutin, flavonoids, and antioxidant activity (Liu et al., 2016). Among the numerous abiotic factors that influence secondary metabolism in plants, ultraviolet (UV) radiation is particularly impactful. For instance, studies on *Prunella vulgaris* revealed that exposure to UV-B radiation led to an increased production of total flavonoids, rosmarinic acid, and caffeic acid. Similarly, *N. humboldtiana* responded to elevated UV radiation by enhancing its antioxidant activity, which was linked to an increase in flavonoid production and an overall improvement in photosynthetic potential (Nocchi et al., 2020; Pant et al., 2021). Furthermore, the level of biologically active compounds in plants is subject to fluctuations induced by a variety of factors, including the plant species and cultivar, the developmental stage of the plant, and the characteristics of the soil (such as its chemical composition and physical properties). Additional factors influencing the concentration of active substances include the level of irrigation, atmospheric concentrations of CO_2 and O_3, as well as agronomic practices such as the application of fertilizers and irrigation management. These factors collectively affect the growth and development of plants, leading to variations in the composition and concentration of biologically active compounds (Boretti and Florentine, 2019; Pant et al., 2021).

The objective of this study is to evaluate the impact of fertilization on the growth and development of *Salvia sclarea L.* (clary sage). In this experiment, the biopreparation TERIA-S, a bacterial-based fertilizer, was employed. This biopreparation was isolated from highly saline soils in the Kungrad district of the Republic of Karakalpakstan and is characterized by its bacterial origin. The bioactivator contains microorganisms from the strains Bacillus subtilis K-4, Bacillus thuringiensis K-7, and Priestia megaterium K-8. These microorganisms exhibit a broad range of polyfunctional properties that benefit both the soil and plants. Specifically, they possess phytohormonal and growth-stimulating activities, facilitate the conversion of microelements into forms that are readily assimilable by plants, neutralize the alkaline pH of saline soils, and enhance the accumulation of heavy metals within the soil matrix (Hassan et al., 2017).

In Uzbekistan, the TERIA-S bioactivator has been widely used for the cultivation of various agricultural crops, including seed-sprouted plants, potato tubers, and tree and shrub seedlings. However, its application in the cultivation of medicinal herbs remains unexplored. The primary aim of utilizing the bacterial fertilizer in this study is to assess its effects on the external morphological indicators of clary sage, as well as to investigate how the treatment influences the plant's resistance to abiotic stresses, such as drought and climatic fluctuations.

RESEARCH METHODS

The systematic implementation of research methods follows a structured sequence to ensure the rigor, validity, and reliability of the study. In this study, the sequence includes the following steps, which are shown in Figure 1.

Microbiological and agrochemical soil analysis

Microbiological analyses of the soils were conducted using standard methods of soil microbiology (Philippot et al., 2012). To determine the abundance of ammonifiers, oligonitrophils, phosphorus-mobilizing bacteria, nitrogen fixers, micromycetes, and actinomycetes, the studies were carried out on solid agar-based elective nutrient media in four distinct stages:

- preparation of a series of dilutions of the soil suspension;
- inoculation of the dilutions onto agar elective nutrient media;
- incubation of the inoculated media in a thermostat at a temperature of 28–30 °C;
- enumeration of the number of colonies that grew on the solid nutrient elective media.

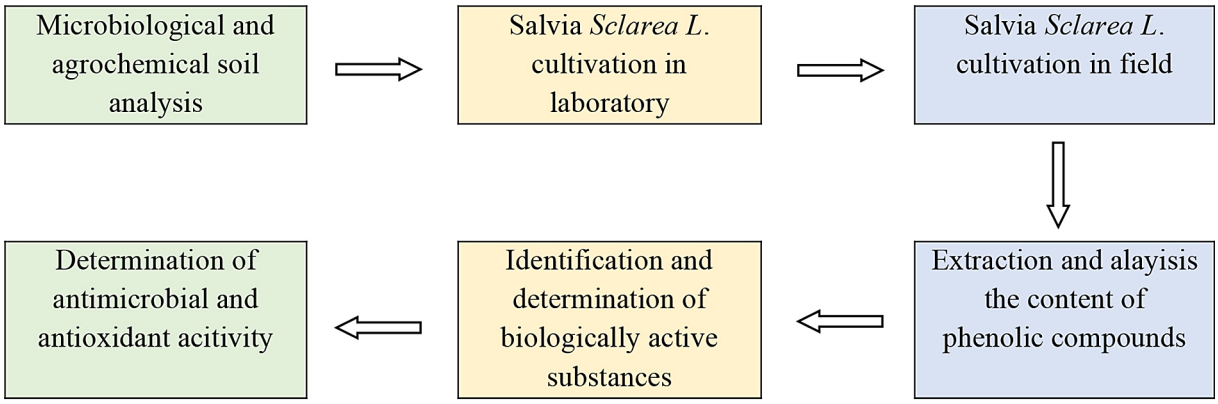


Figure 1. Sequence of implementation of research methods

The following selective nutrient media were utilized for the microbiological analysis of soils: Fish Peptone medium for ammonifiers, Ashby medium for oligonitrophils and nitrogen-fixing bacteria, Pikovskaya medium for phosphorus-mobilizing bacteria, and Chapek medium for micromycetes and actinomycetes.

For the agrochemical analysis, a soil sample was collected from an elevation of 1500 meters in the Kashkadarya region, near the city of Kitab. The agrochemical analysis of the soil was conducted using widely accepted methodologies in agrochemistry. The determination of soil salinity under laboratory conditions was performed through the analysis of water extracts (Ouzemou et al., 2025). This method involves evaluating the content of water-extractable salts (WES) present in the soil. Both cations and anions were quantified gravimetrically. Soil salinity was assessed by measuring the electrical conductivity of soil suspensions prepared at a soil-to-water ratio of 1:1 (EC 1:1). Electrical conductivity was measured in decisiemens per meter (dS/m) using an electrical conductivity meter, which was equipped with a temperature-compensating electrode to account for temperature variations during measurement.

The method of assessing soil salinization through the electrical conductivity of saturated soil extracts (ECe) is widely accepted in international practice due to its simplicity; however, it has not been extensively applied in local conditions. Through a series of representative measurements of both ECe and EC 1:1, a conversion factor (K) of 3.5 was established for the specific conditions of Uzbekistan. This factor enables the assessment of soil salinity, based on measured values of EC in a 1:1 suspension, using the salinity classification system of the Food and Agriculture Organization (FAO). The conversion from EC 1:1 to ECe is recommended to be performed using the following formula (Equation 1):

$$Ece = 3.5 \times EC1{:}1 ECe = 3.5 \times EC1{:}1 \quad (1)$$

Subsequently, the degree of soil salinity can be evaluated for soil layers at depths of 0–30 cm, 30–70 cm, and 70–100 cm according to the classification provided in Table 1.

The method for calculating the content of toxic salts consisted of determining hypothetical salts in mg-eq/100 g of soil.

Laboratory study using cultivation of *Salvia sclarea* L.

Laboratory experiment was conducted according to the methods of setting up laboratory experiments with plants. The laboratory experiment with clary sage was set up in pots with a volume of 1.6 l with soil (1 kg) in 5 variants according to the following scheme (Table 2).

Ammophos fertilizer was added to the soil at a rate of 20 g per 1 kg of soil (1 vessel). The plant seeds and soil were treated with TERIA-S bacterial fertilizer with a cell titer of 6 × 108 CFU/ml. The products were diluted with running cold water. A single amount of clary sage (2.5824 g) was planted in each pot.

Salvia sclarea L. cultivation in the field

Clary sage (*Salvia sclarea L.*) was cultivated in an agricultural setting using seeds treated with a bioactivator. Prior to planting, the soil in the plot was prepared through a series of pre-sowing treatments, including loosening, surface leveling, and the removal of weed seedlings. Ten raised

Table 1. Soil salinity assessment according to FAO

Degree of salinity	Ece, dSs/m
Non-saline soils	0–2
Slightly saline soils	2–4
Moderately saline soils	4–8
Highly saline soils	8–16
Very highly saline soils	> 16

beds, each measuring 3 meters in length and 3 meters in width, were established on the plot. The seeds were evenly distributed across the beds, with a standardized amount of seeds (one level teaspoon) applied to each row.

To evaluate the effect of the bioactivator on flavonoid content and other relevant parameters, six of the ten rows were treated with the bioactivator. The seeds in these rows were subjected to a dry spray treatment with the bioactivator after the seeds had been evenly distributed. Four rows were designated as the control group, where no bioactivator treatment was applied. Irrigation was conducted as needed, based on prevailing weather conditions.

Upon the completion of the vegetative period, two specimens of clary sage – one from the control group and one from the bioactivator-treated group – were harvested separately. The plants were then dried in a well-ventilated room, shielded from direct sunlight, to ensure proper preservation for further analysis.

Extraction and analysis the content of phenolic compounds in *Salvia sclarea* L.

The content of intracellular phenolic compounds in the extracts was calculated using the following formula (Equation 2):

$$F = \frac{C_F \cdot V_Э}{m \cdot (1-W) \cdot 1000} \quad (2)$$

where: F – total content of intracellular phenolic compounds, mg-eq gallic acid/g dry weight; C_F – concentration of phenolic compounds calculated using the calibration curve based on the optical density of reaction mixtures, mg-eq gallic acid/l; V_E – total volume of extract, ml; m – weight of sample, g; W – raw material moisture content, fractions; 1000 – conversion factor from l to ml.

Table 2. Scheme of laboratory experiment with *Salvia sclarea* L.

No.	Scheme of soil and seed processing
1	Control - no fertilizers, dry seeds
2	Control – with ammophos fertilizer, seeds moistened with water
3	Practice - seeds treated with bacterial fertilizer TERIA-S
4	Practice - with ammophos fertilizer, seeds treated with bacterial fertilizer TERIA-S
5	Practice - soil and seed treatment with bacterial fertilizer TERIA-S

Following the drying and grinding of the above-ground parts of clary sage, the extraction process was carried out using ethyl alcohol. During the course of the study, optimal conditions for the extraction of flavonoids from sage were determined, including the concentration of the aqueous-alcoholic ethyl alcohol solution, as well as the time and temperature parameters for the extraction process. These variables were carefully selected to maximize the yield and efficiency of flavonoid extraction from the plant material (Tables 3, 4 and 5).

In conclusion, the optimal parameters for the extraction of flavonoids from *Salvia sclarea* L. were determined as follows: a 70% aqueous-alcoholic solution of ethyl alcohol, an extraction duration of 30 minutes, and an extraction temperature of 70 °C. These conditions were identified as the most effective for maximizing the yield of flavonoids from the plant material.

Identification and determination of biologically active substances

Chromatographic determination of kaempferol-3-β-D-glucopyranoside in clary sage extracts was performed by chromatograph mass spectrometry on a gas chromatograph mass spectrometer (Waters, USA) equipped with a Symmetry C18 250 × 4.6 mm, 5 µm column (Waters, USA). The volume of the injected sample was 1 µl. A standard solution of the commercial preparation kaempferol-3-β-D-glucopyranoside (Sigma, France) was used to determine kaempferol-3-β-D-glucopyranoside.

Mass spectra were recorded in the negative and positive ion ranges. Mass spectrometry parameters in the negative ion range were: capillary voltage – 3 kV, cone voltage – 30 V, extractor voltage – 4 V; in the positive ion range: capillary

Table 3. Dependence of the amount of flavonoids on the concentration of the aqueous-alcoholic solution of ethyl alcohol

Concentration of aqueous-alcoholic solution of ethyl alcohol	Flavonoid content, mg-eq rutin/g absolutely dry raw material
30%	58.07±0.74
50%	61.83±0.59
70%	61.52±0.64
Note: extraction parameters: raw material: extractant = 1:30, T = 70 °C, t = 30 min.	

Table 4. Dependence of the amount of flavonoids on the extraction time

Extraction time	Flavonoid content, mg eq. rutin/g absolutely dry raw material
30 мин	61.83±0.38
45 мин	61.92±0.52
60 мин	62.98±0.46
Note: extraction parameters: CCH_2OH = 70%, raw material: extractant = 1:30, T = 70 °C.	

Table 5. Dependence of the amount of flavonoids on the extraction temperature

Extraction temperature	Flavonoid content, mg eq. rutin/g absolutely dry raw material
50 °C	54.73±0.57
70 °C	61.83±0.36
82.51 °C ($T_{boiling}$)	58.89±0.58
Note: extraction parameters: CCH_2OH = 70%, t = 30 min, raw material:extractant = 1:30.	

voltage – 3 kV, cone voltage – 40 V, extractor voltage – 3 V. Desolvation temperature was 400 °C, source temperature was 130 °C, and total inert gas (nitrogen) consumption was 480 l/h. Chromatographic separation was monitored using a diode array detector in the wavelength range of 200–790 nm and an electrospray ionization (ESI) mass detector.

The process for fractional separation of biologically active substances in the tincture of *Salvia sclarea* included the concentration of the alcohol-water extract, followed by sequential liquid-liquid extraction with solvents of increasing polarity. In this experiment, ethyl acetate and butanol were selected as solvents for the extraction process. Subsequently, phenolic compounds present in the ethyl acetate, butanol, and ethanol extracts were analyzed using HPLC with a Shimadzu LC-20AD system (Japan), equipped with a manual injector. The data obtained were processed using the Multichrome for Windows software.

The stationary phase consisted of a Kromasil C18 column, with a particle size of 5 μm and dimensions of 4.6 × 250 mm. Detection of the compounds was performed using a Shimadzu SPD-M20A diode-array detector (Japan), with a D2/W radiation source and a detection wavelength of 270 nm. A gradient elution system was employed, using solvent A (methanol: distilled water, 10:15, v/v) and solvent B (distilled water) as the mobile phase at a flow rate of 1 ml/min. The column temperature was maintained at 28 °C, and the injection volume was 10 μL.

Determination of antimicrobial and antioxidant activity of the sum of phenolic compounds

The free radical scavenging activity was assessed using the DPPH assay, as described by Shimada et al. A 0.1 mM DPPH solution was prepared by dissolving 3.94 mg of DPPH in 100 mL of ethanol. Subsequently, 1 mL of this DPPH solution was added to 3 mL of different sample concentrations (250, 125, 62.5, 31.25, and 15.62 μg/mL). An equivalent amount of ethanol was used in the control test. The mixtures were vigorously shaken, well-mixed, and allowed to stand at room temperature for 30 minutes. After the incubation period, the absorbance of each sample was measured at 517 nm using a UV-Vis spectrophotometer. The DPPH radical inhibition

percentage (%I) was calculated using the following formula (Equation 3):

$$Inhibition\ (\%) = (AA - B) \times 100 \quad (3)$$

where: *A* represents the absorbance value of the control, *B* denotes the absorbance value of the sample.

Antimicrobial activity was evaluated using the well-diffusion agar method, with slight modifications. The bacterial strains used were *Staphylococcus aureus*, *Bacillus subtilis* (Gram-positive), *Pseudomonas aeruginosa*, and *Escherichia coli* (Gram-negative). Bacterial suspensions were prepared to a titer of 1.5×10^8 CFU/mL according to the McFarland standard. 0.5 mL aliquot of each bacterial suspension was spread on the surface of meat-peptone agar (MPA) plates and incubated for 15 minutes. The extracts were then diluted in dimethyl sulfoxide (DMSO) to a concentration of 50 mg/mL. A volume of 100 µL of each sample was introduced into wells created in the agar. Following incubation at 37 °C for 24 hours, the inhibition zone was measured. Each experiment was performed in triplicate to ensure reproducibility of results.

RESULTS AND DISCUSSION

Microbiological and agrochemical soil analysis

The microbiological analysis of the original soil indicated that populations of ammonifying bacteria, oligonitrophilic microorganisms, micromycetes, and actinomycetes were within normal ranges. In contrast, nitrogen-fixing bacteria were present at levels 2–3 orders of magnitude below the typical values, and phosphate-mobilizing bacteria were not detected. Under natural conditions, these microorganisms facilitate the conversion of phosphorus-containing molecules into a soluble form (Cheng, et al., 2023). It is widely known that phosphorus is a key macronutrient required for proper plant nutrition. It enhances important physiological and biochemical processes, increases temperature tolerance, and promotes the plant's resilience to diverse abiotic stressors. Insufficient phosphorus availability in the soil has a negative impact on plant growth and development, as well as a considerable reduction in resistance to pathogens (Ibrahim et al., 2022).

Thus, based on the obtained data, it can be concluded that plants will completely absorb carbon and proteins from the soil, and partially nitrogen, but plants will not fully absorb phosphorus from soil reserves. Consequently, the study examined the utilization of phosphorus-mobilizing bacterias and in the corporation of supplementary phosphorus fertilizer, specifically ammophos, into the soil (Table 6).

From the obtained data on the agrochemical composition of the soil, it can be concluded that the soils are non-saline chloride-sulphate type, strongly alkaline (pH = 8.7) (Table 7). Plants of the Lamiaceae family generally exhibit a preference for soils with a pH range of 6.0 to 8.0 and are not highly demanding with respect to soil type. Several studies have demonstrated that an increase in soil pH within this range may enhance the biosynthesis of secondary metabolites in species belonging to this family (Beigi, 1995). Specifically, research on *Thymus pubescens* has shown that soils with a pH between 6 and 8 positively influence essential oil yield (Yousef et al., 2016). Therefore, while the recorded soil pH of 8.7 slightly exceeds the optimal range, it may still be considered partially favorable for the cultivation of clary sage (*Salvia sclarea*), particularly given the species' tolerance for mildly alkaline conditions.

Soil was identified as rich in humus (2.07%), moderately supplied with nitrogen, rich in phosphorus and very poor in potassium, low in microelements (Ca, Mg, Na, K) (see Table 7.). In the original soils, the molybdenum content was 10 mg / kg, which does not exceed the MAC in gray soils of Uzbekistan (SanPiN RUz N 0055-96). The boron content was 20 mg/kg, this means that the original soils are rich in boron. Therefore,

Table 6. Microbiological analysis of the original soil

Original soil	Number of microorganisms, CFU/1 g of soil					
	Ammonifiers	Oligonitrophils	Nitrogen fixers	Phosphate-solubilizing bacteria	Micromycetes	Actinomycetes
Rate	7.5×10^8	2.8×10^7	1.5×10^3	Not found	2.0×10^3	4.3×10^4
	$n \times 10^8$	$n \times 10^7$	$n \times 10^{5-6}$	$n \times 10^{7-8}$	$n \times 10^{2-3}$	$n \times 10^{4-5}$

there is no need for additional introduction of B and Mo into these soils. The content of toxic salts was determined by recalculating the data obtained from water extract analyses. The results of converting individual ions into their corresponding hypothetical salts are presented in Table 7. Based on these recalculations, it was found that the proportion of toxic salts in the soil exceeded that of non-toxic salts by 0.017%. Among toxic salts, sodium sulfate occupies the largest share, more than 50% of the total toxic salts.

Using bacterial fertilizer in a lab experiment

Approximately one week following planting, initial seedling emergence was observed. However, germination was inconsistent across the experimental pots, with some failing to produce any shoots. A comprehensive comparative analysis of plant development was conducted one month after planting. The results, presented in Figure 2, revealed clear differences between the experimental conditions. In control Group 1, incomplete germination was evident, indicating that not all seeds successfully sprouted. Experimental Groups 2 and 4, both of which involved the application of ammonium phosphate (amphos), did not exhibit seedling emergence, even when supplemented with bioactivators. In contrast, Group 3 demonstrated high germination rates and produced seedlings of superior quality. Group 5 was notable for producing taller seedlings; however, these plants exhibited poor structural integrity and weak morphological development (Figure 2).

In laboratory plantings of clary sage, experiments where ammophos was used did not produce shoots (2, 4). Ammophos is recognized as one of the most effective phosphorus-based fertilizers, suitable for application at any stage of the growing season and across a wide range of soil types. It is particularly utilized in regions and climatic zones where crops exhibit a high demand for phosphorus and a relatively low requirement for nitrogen. According to findings from the Russian Research Institute of Agriculture, the application of ammophos on neutral and alkaline soils has been shown to increase crop yields by an average of 76%. Despite its demonstrated efficacy, excessive application of ammophos can be detrimental to plant development, with potential negative effects surpassing those associated with phosphorus deficiency (Akanova et al., 2022). In the present study, the application of ammophos at a rate of 20 g per 5 kg of soil adversely affected seed

Table 7. Agrochemical soil analysis

Assessment of the degree of salinity according to ECE, type of salinity, dS/m	pH	Indicator
Chloride-sulphate type soil, 0.48 dS/m	8.7	Strongly alkaline
Humus in soil (norm %, 0.91-1.35)	Humus carbon, % (Cr, %)	Assessment of humus supply
1.07	0.92	mid
Plant-absorbable forms of N-NO_3 in soil (30-50 mg/kg)	Gross forms of nitrogen in soil % (norm 0.09-0.12)	Assessment of nitrogen supply
62.0	0.088	mid
Plant-absorbable forms of P_2O_5 (norm 46-60 mg/kg)	Gross forms of phosphorus in this soil (norm % 0.21-0.26)	Assessment of phosphorus supply
0.112	27.0	low
forms of K_2O assimilated by plants (norm 301-400mg/kg)	Gross forms of potassium in this soil (norm % 1.8-2.4)	Assessment of potassium supply
0.69	120	Mid

Content of soluble ions, %										
EC 1:5 dS/m	ECe, dS/m	Dense residue, %	HCO_3	Cl	SO_4	Ca	Mg	Na	K	Sum of ions, %
0.28	1.12	0.114	0.033	0.007	0.048	0.014	0.004	0.015	0.003	0.107

Salt content, %								
$Ca(HCO_3)_2$	$CaSO_4$	$MgSO_4$	Na_2SO_4	NaCl	$MgCl_2$	Sum of non-toxic salts, %	Sum of toxic salts, %	Sum of salts, %
0.022	0.029	0.018	0.038	0.012	-	0.051	0.068	0.118

Figure 2. *Salvia Sclarea* L. seed growth and development

germination, which may be attributed to an overly high concentration of the active component.

Three months after planting, the effect of the bacterial fertilizer TERIA-S on the biometric indicators of growth and development of *Salvia sclarea* L. was assessed. Two experimental variants were selected: the control group (variant 1) and the experimental group (variant 3), the latter of which included the treatment of *Salvia sclarea* L. seeds with bacterial fertilizer. The results of the study showed that the total height of seedlings in the experimental group (variant 3), treated with the biofertilizer TERIA-S, was statistically significantly greater than in the control group (variant 1) (Table 8 and Figure 3). The results of the conducted studies demonstrate that the application of biofertilizers exerts a positive effect on all growth parameters of *Salvia sclarea* L. seedlings. Specifically, the average height of seedlings treated with TERIA-S was 4.9 cm greater than that of the control group, and root length increased by an average of 4.1 cm. The height of the above-ground part of the seedlings showed minimal variation, being only 8 mm greater in the biofertilizer-treated group compared to the control. In terms of biomass, the average weight of seedlings in the control group was 948 mg, while the application of the bacterial fertilizer resulted in an average increase to 1120 mg. The weight of the above-ground part did not differ significantly, with a difference of approximately 40 mg in favor of the biofertilizer-treated variant. However, root weight was more than 2.2 times greater in the biofertilizer-treated seedlings compared to the control, suggesting a significantly enhanced root system development.

The results of the conducted studies demonstrate that the application of biofertilizers exerts a positive effect on all growth parameters of *Salvia sclarea* L. seedlings. Specifically, the average height of seedlings treated with TERIA-S was 4.9 cm greater than that of the control group, and root length increased by an average of 4.1cm. The height of the above-ground part of the seedlings showed minimal variation, being only 8mm greater in the biofertilizer-treated group compared to the control. In terms of biomass, the average weight of seedlings in the control group was 948 mg, while the application of the bacterial fertilizer resulted in an average increase to 1120 mg. The weight of the above-ground part did not differ significantly, with a

Table 8. Effect of bacterial fertilizer TERIA-S on biometric growth indices and weight of clary sage (after 3 months of laboratory experiment)

Total height of sprout (cm)		Root length (cm)		Height of the above-ground part of the shoot (cm)	
Control	Seed treatment with TERIA-S	Control	Seed treatment with TERIA-S	Control	Seed treatment with TERIA-S
16.8±0.07	21.7±0.56*	8.6±0.2	12.7±0.24*	8.2±1.12	9.0±0.32
* P≤0.05 –reliable relative to control					
Total weight of sprout, (mg)		Root weight, (mg)		Weight of the aboveground part of the sprout, (mg)	
Control	Seed treatment with TERIA-S	Control	Seed treatment with TERIA-S	Control	Seed treatment with TERIA-S
948±2.7	1120.0±4.4*	113.0±2.8	247.0±3.6*	835.0±4.0	873.0±3.6*
*P≤0.05 – reliable relative to control					

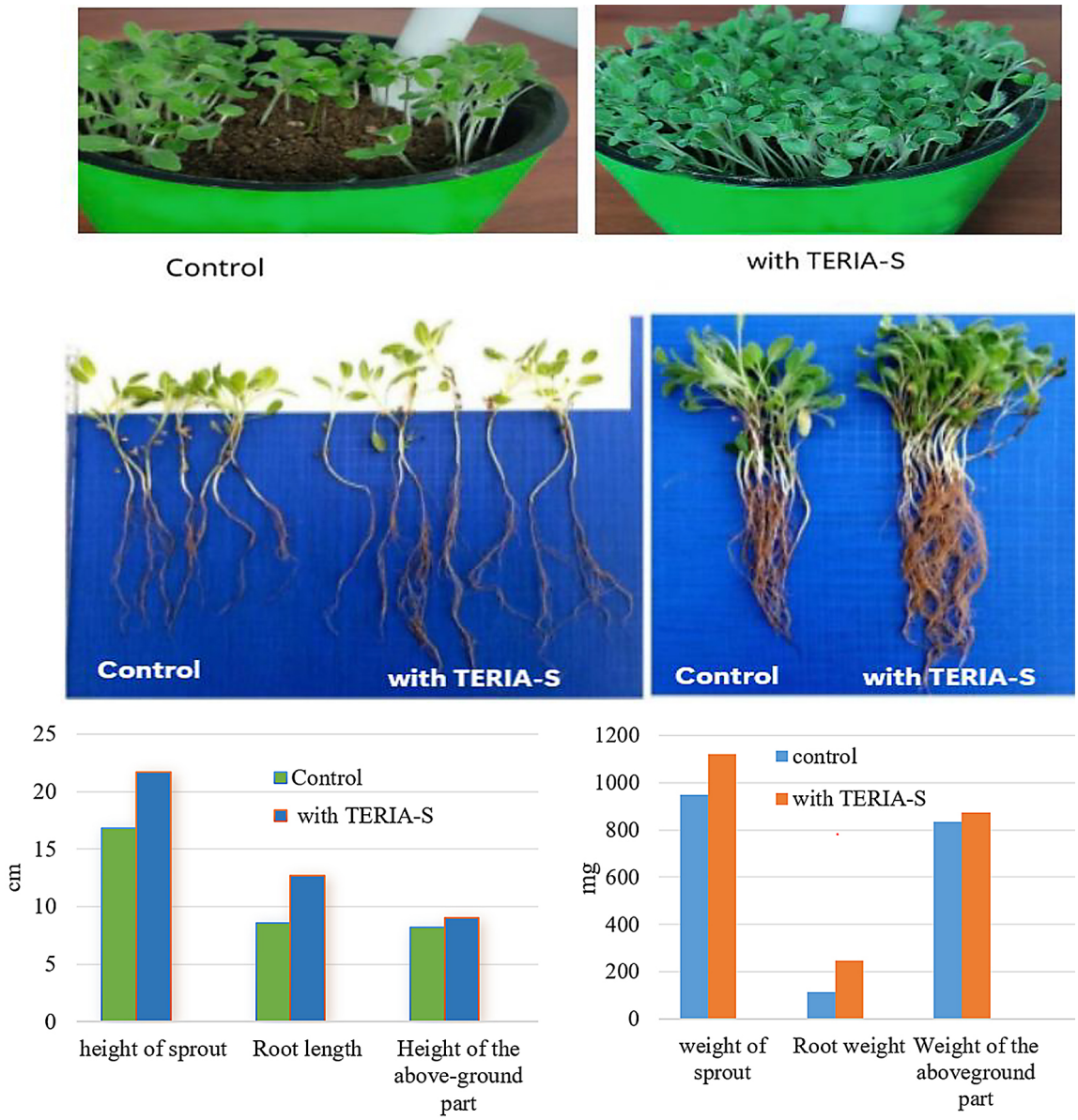


Figure 3. Effect of bacterial fertilizer TERIA-S on biometric indicators of growth and development of sage (after 3 months of sowing)

difference of approximately 40 mg in favor of the biofertilizer-treated variant. However, root weight was more than 2.2 times greater in the biofertilizer-treated seedlings compared to the control, suggesting a significantly enhanced root system development. This improvement in seedling growth can be attributed to the beneficial microorganisms present in the biofertilizer, which during their metabolic activity release biologically active substances such as growth regulators, hydrolytic enzymes, amino acids, vitamins, and antimicrobial agents. These compounds contribute to the activation of growth processes at the early stages of ontogenesis, thereby creating more favorable conditions for seedling emergence and development (Stamenković et al., 2018).

There are a significant number of studies confirming the effectiveness of microbiological fertilizers (Basu et al., 2017; Finkel et al., 2017; Vasilchenko et al., 2021). For instance, Chekaev et al. reported that microbiological preparations significantly enhanced the germination energy of spring wheat seeds, increasing it to 96–100%, compared to 92% in the control group. Laboratory germination rates were either comparable to or slightly higher than the germination energy.

Based on the data obtained, it can be concluded that bacterial fertilizers show promising results in pre-sowing treatment of *Salvia sclarea* L. under

laboratory conditions, indicating their potential for application in field conditions (Figure 3).

Cultivation of clary sage in the field

At the conclusion of the growing season, *Salvia sclarea* L. plants were harvested in their entirety, including both the aerial parts and root systems. A comparative analysis was subsequently conducted to evaluate the lengths of the aboveground biomass and root structures (see Figure 4 and Table 9). Plant samples were collected using the checkerboard sampling method to ensure representative data. For the purpose of analyzing the aerial components of *Salvia sclarea* L., the root systems were carefully separated from the green biomass prior to measurement.

The stimulatory effects of the *Bacillus subtilis K-4, Bacillus thuringiensis K-7,* and *Priestia megaterium K-8* strains were evident from the early stages of clary sage development, beginning with seed germination. Pre-sowing treatment of *Salvia sclare* L. seeds with the bioactivator resulted in a 1.3-fold increase in sprout stem length and a 1.6-fold increase in root length compared to the untreated control. As shown in Table 9, the bacterial strains had a pronounced influence on root development. The treated plants exhibited more extensively branched root systems that penetrated deeper into the soil relative to the control group.

Given that the biopreparation exerted a stronger effect on root system development, it can be concluded that the bioactivator demonstrates significant potential for promoting plant growth, particularly in root crops, and may be a valuable tool for enhancing the productivity of agricultural systems. Shakina, when using bacterial fertilizers on agricultural crops, showed good results on the growth and development of onions and table

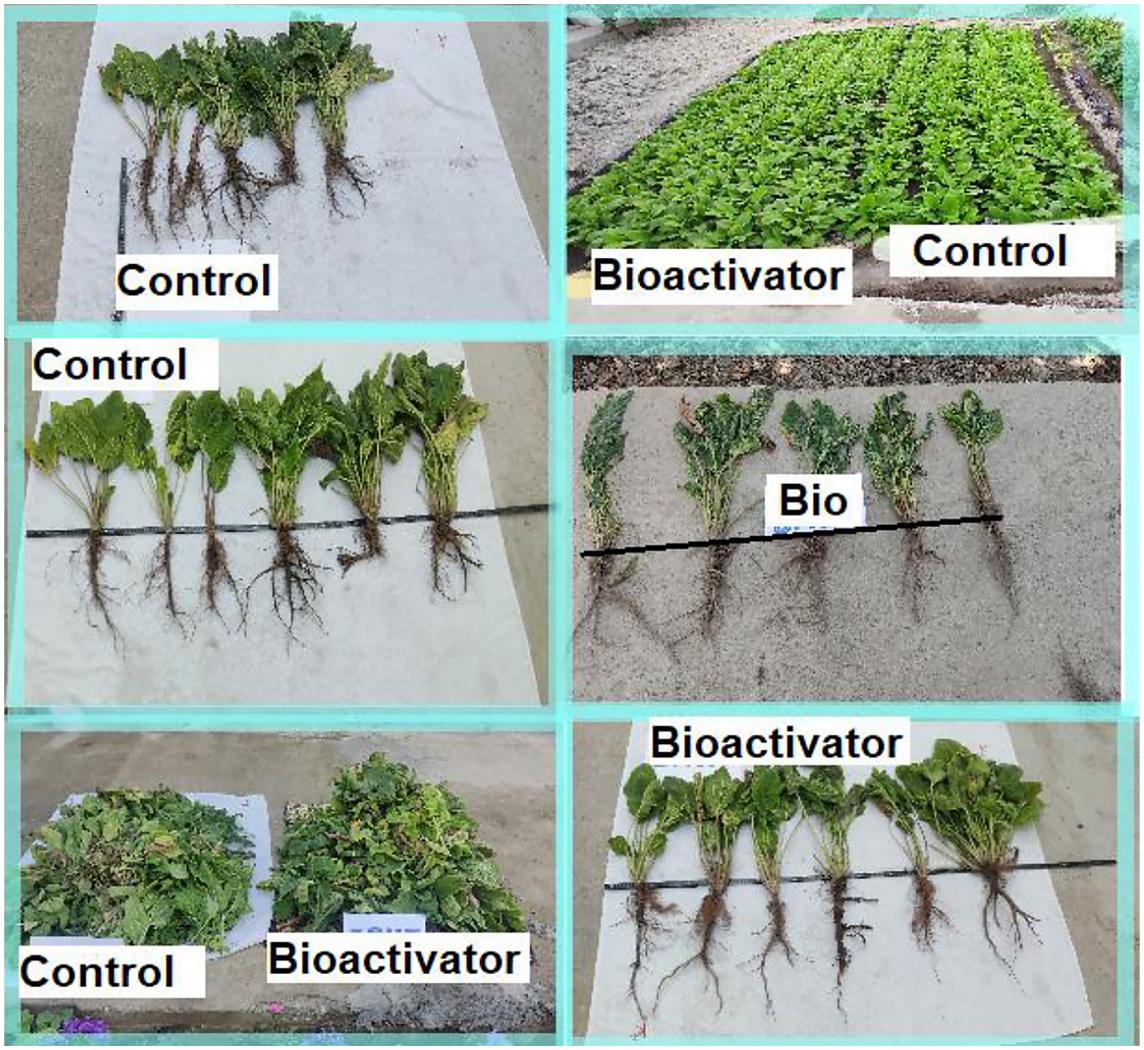


Figure 4. The process of harvesting clary sage in the field

Table 9. Effect of bioactivator on growth parameters of *Salvia sclarea* L. in field conditions

Sample number		1	2	3	4	5	6	7	8	9	10	11	Average
Control	Stems/cm	20	16	14	19	10	12	15	14	13	15	8	14±1.5
	Roots/cm	12	10	9	11	7	10	15	13	11	11	7	10.5±1.5
TERIA-S	Stems/cm	20	21	19	20	20	16	19	20	15	24	16	19±1.5
	Roots/cm	16	15	16	20	19	15	14	19	15	22	15	17±1.5

carrots, since the use of a preparation based on agrobacteria increased the absorption activity of the roots (Shakin et al., 2006).

Study of the content of phenolic compounds

In the initial phase of the study, the intracellular content of phenolic compounds and flavonoids in clary sage (*Salvia sclarea*) samples was quantified. The results are summarized in Table 10. The concentration of phenolic compounds was expressed in milligrams of gallic acid equivalents (mg GAE) per gram of absolutely dry raw material. Analysis of the data revealed that the phenolic content in sage leaf blades amounted to 34.74 ± 1.12 mg GAE/g of absolutely dry raw material, while the flavonoid content, expressed in milligrams of rutin equivalents (mg RE), was 37.46 ± 1.48 mg RE/g of absolutely dry raw material.

Identification and determination of biologically active compounds

Identification of individual compounds by means mass and electronic spectrometry revealed that the peak with a retention time of 10.76 minutes corresponds to kaempferol-3-β-D-glucopyranoside. In the positive ion mode mass spectrum (Figure 5a), a molecular ion at m/z 449.61 was detected, corresponding to $[M+H]^+$, which represents the protonated molecule of kaempferol-3-β-D-glucopyranoside. Additionally, a fragment ion at m/z 287.53 was observed, corresponding to $[M–glu+H]^+$, indicative of the kaempferol aglycone. In the negative ion mode (Figure 5b), the spectrum exhibited a molecular ion at m/z 447.68 ($[M–H]^-$), as well as a fragment ion at m/z 285.61 ($[M–glu–H]^-$), further confirming the identity of the compound as kaempferol-3-β-D-glucopyranoside.

High-performance liquid chromatography (HPLC) analysis of phenolic compounds was performed using a UV detector set at a wavelength of 254 nm. It is important to note, however, that not all flavonoids and phenolic acids exhibit absorption maxima at this wavelength, which may influence the detection sensitivity for certain compounds. The analysis was conducted on ethanol extracts obtained from clary sage (*Salvia sclarea*) samples, both untreated (control) and treated with a bioactivator. For comparative purposes, a standard for phenolic compounds was employed to assess the composition of the phenolic profile in the above-ground parts of clary sage at 254 nm (Figure 6) (Gavrilin et al., 2010). Among the phenolic compounds at wave 254, 6 phenolic acids (gallic acid, chlorogenic acid, caffeic acid, chicoric acid, ferulic acid, and rosmarinic acid) and 6 representatives of flavonoids were found, one of the tannins, as well as umbelliferone, and coumarin from the coumarin group.

It is well established that polar compounds exhibit greater solubility in polar solvents due to their high dielectric constants, whereas non-polar substances are more soluble in non-polar solvents, which possess lower dielectric constants (Nuno et al., 2019). This principle underpins the strategy of sequential solvent extraction, wherein raw plant materials are treated with solvents of increasing or varying polarity to yield distinct fractions, each enriched with different physiologically active constituents. High-performance liquid chromatography (HPLC) serves as an effective analytical technique for quantifying the content

Table 10. Results of studies of the content of intracellular phenolic compounds and flavonoids

Sample	Object of study	Content of intracellular phenolic compounds, mg-eq gallic acid/g absolutely dry raw material	Flavonoid content, mg-eq rutting/g absolutely dry raw material
Salvia sclarea L.	Leaf blades	34.74±1.12	37.46±1.48

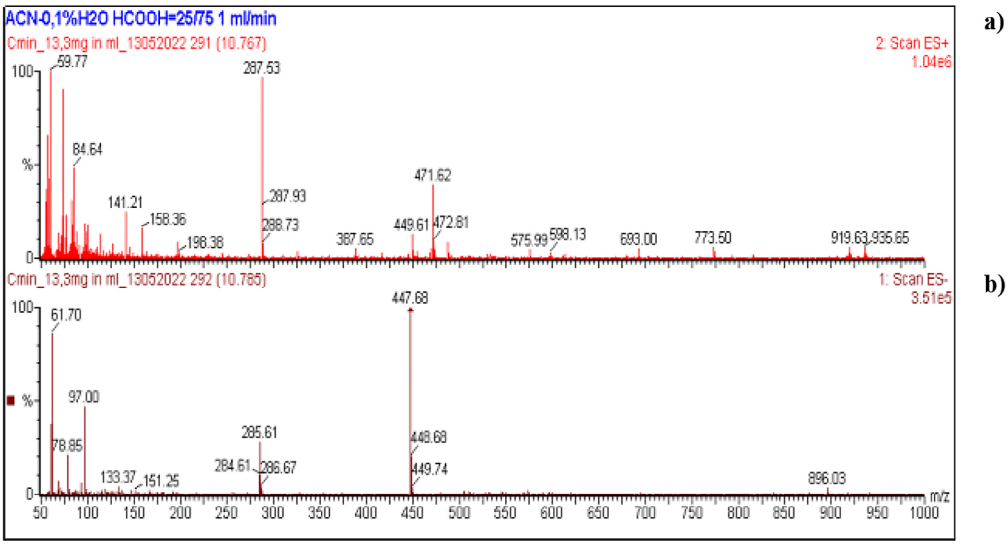


Figure 5. The mass spectrum of the compound with a retention time of 10.76 minutes: a) positive ion location, b) negative ion location

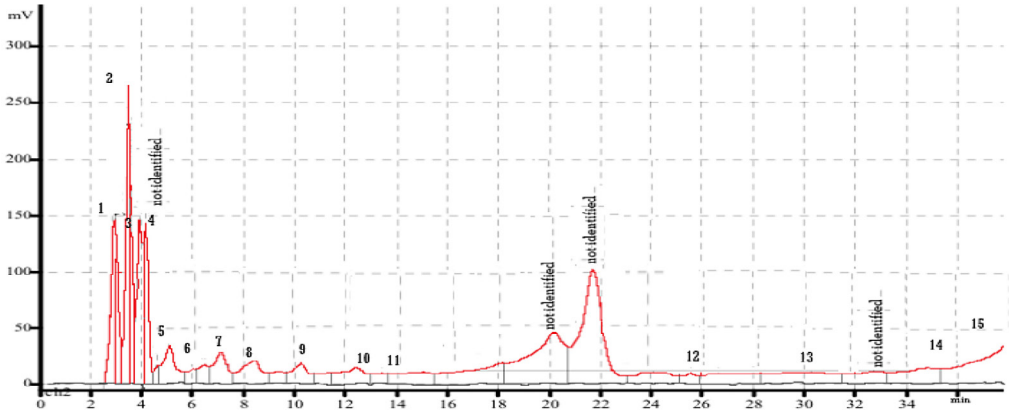


Figure 6. Standard of phenolic compounds found at 254 nm in the aerial parts of *Salvia sclarea* L.: 1 - umbelliferone, 2 - gallic acid, 3 - chlorogenic acid, 4 - tannin, 5 - caffeic acid, 6 - chicory acid, 7 - ferulic acid, 8- routine, 9 - coumarin, 10 - rosemary acid, 11 - luteolin-7-glycoside, 12 - quercetin, 13 - dihydroquercetin, 14 - kaempferol, 15 - apigenin

of various phenolic compound groups within these fractions. In the present study, fractions derived from clary sage (*Salvia sclarea*) were obtained using ethyl acetate, n-butanol, and ethanol as solvents. Each extraction was performed under two conditions: a control and a variant incorporating biofertilizer application. The comparative results of these extractions are illustrated in chromatograms (Figures 7, 8, and 9), enabling a detailed assessment of the influence of both solvent polarity and biofertilizer treatment on the phenolic profile of the plant extracts.

Analysis of the chromatographic data (Table 11) revealed that each solvent extracted a distinct profile of phenolic compounds. Gallic acid, kaempferol, and umbelliferone were consistently present across all fractions, regardless of the extraction method. Chlorogenic acid was detected in all samples except in the ethyl acetate fraction obtained using the biofertilizer treatment. Notably, when compared to the standard reference sample, several phenolic compounds were not detected in the experimental extracts. Among the solvents tested, the most comprehensive extraction was achieved using n-butanol in combination with the biofertilizer TERIA-S, which resulted in the identification of 11 out of 15 target phenolic compounds. In contrast, the control treatment with butanol (i.e., without the biofertilizer) yielded only 5 phenolic compounds. These findings

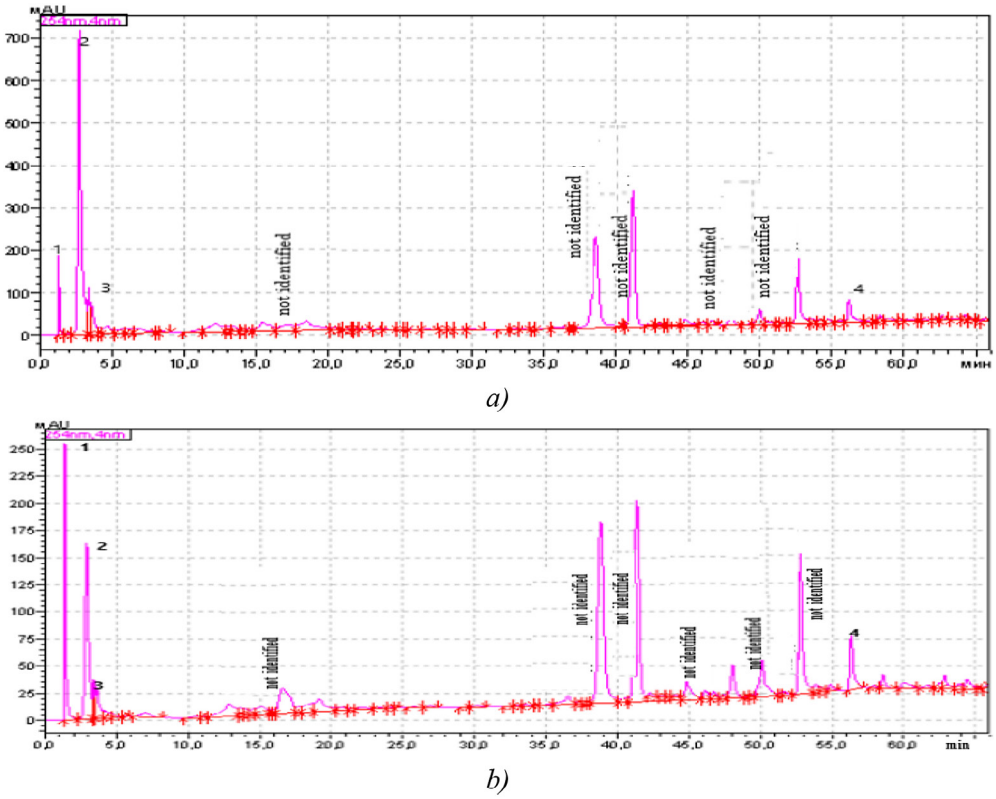


Figure 7. Chromatogram of the ethonic fraction of clary sage: (a) without the use of a bioactivator – control; (b) using a bioactivator 1-Umbelliferone; 2-Gallic acid; 3-Chlorogenic acid; 4-Kaempferol

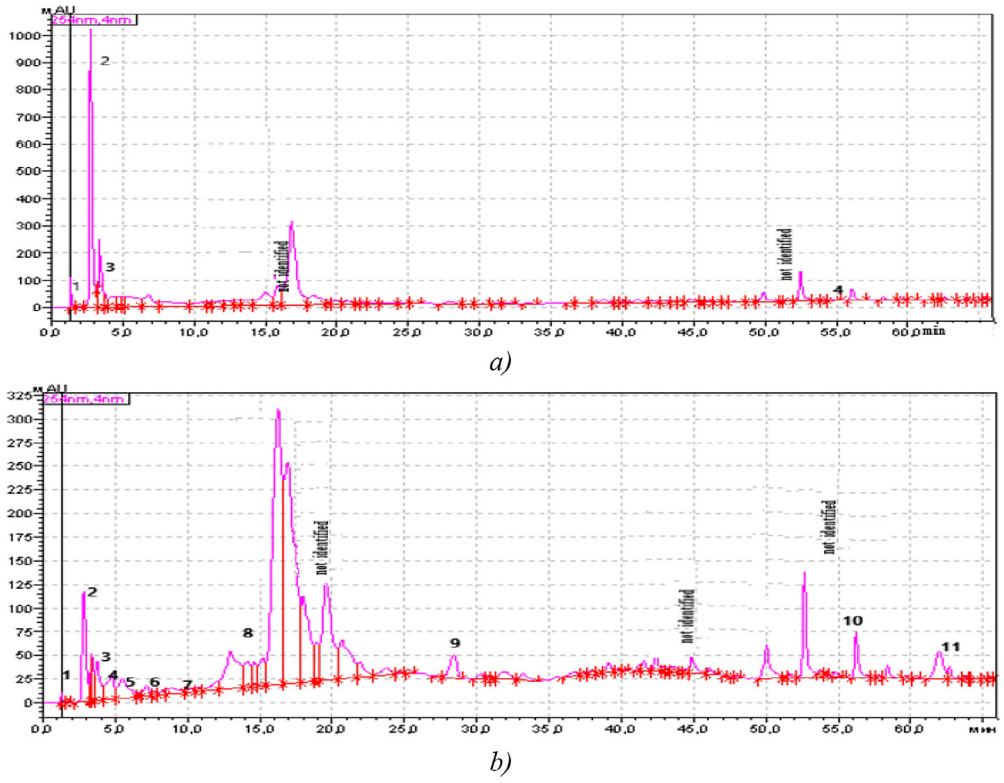


Figure 8. Chromatogram of the butanol fraction of clary sage: (a) without the use of a bioactivator – control, 1-Umbelliferone; 2-Gallic acid; 3-Chlorogenic acid; 4-Kaempferol; (b) with the use of a bioactivator TERIA-S, 1-Umbelliferone; 2-Gallic acid; 3-Chlorogenic acid; 4-tannin; 5-Caffeic acid; 6-Chicoric acid; 7-Ferulic acid; 8-Luteonin -7-glycoside; 9-Quercetin; 10-Kaempferol; 11-Apegenin

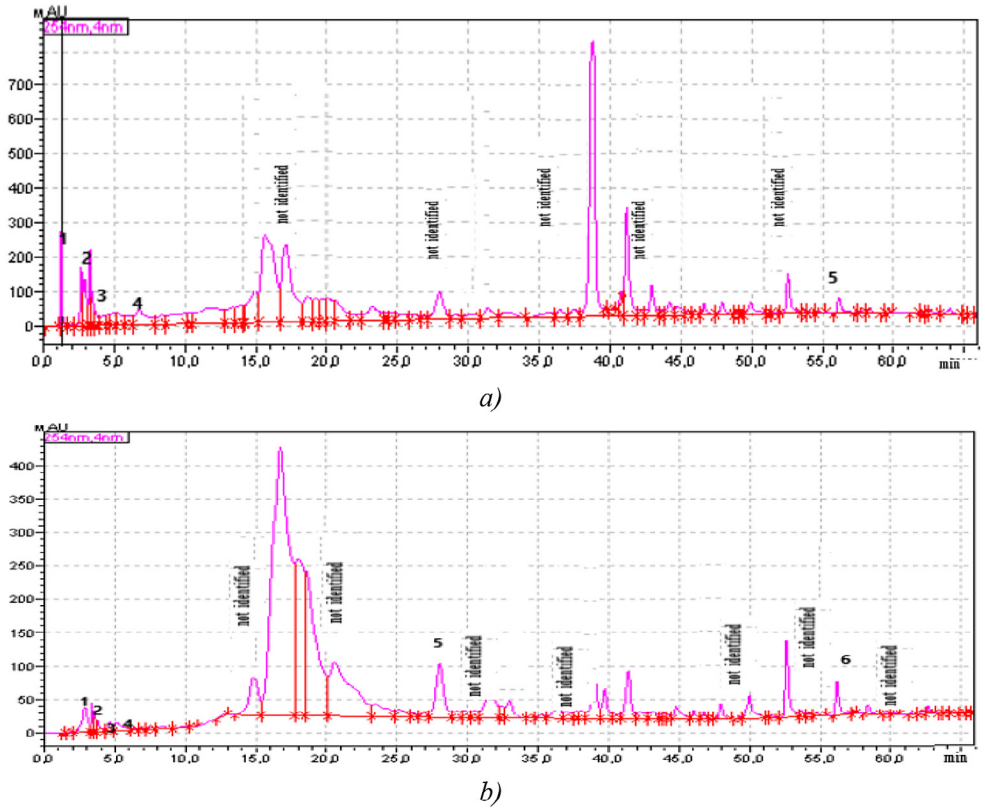


Figure 9. Chromatogram of the ethyl acetate fraction of clary sage: a) without the use of a bioactivator (control) 1-Umbelliferone, 2-Gallic acid, 3-Chlorogenic acid, 4-Ferulic acid, 5-Kaempferol; b) using a bioactivator (TERIA-S) 1-Umbelliferone, 2-Gallic acid, 3-Chlorogenic acid, 4-tannin, 5-Quercetin, 6-Kaempferol

Table 11. Results of HPLC analysis of phenolic compounds of clary sage (254 nm) with three different exctractants

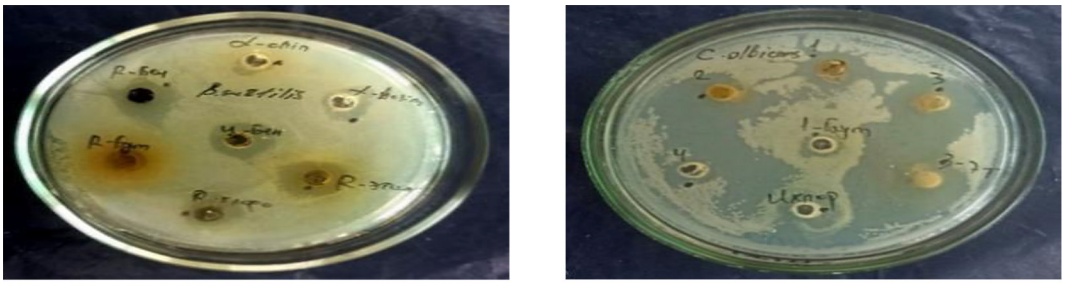
Identified	Retention time, min	Ethanol control	Ethanol TERIA-S	Butanol control	Butanol TERIA-S	Ethyl acetate control	Ethyl acetate TERIA-S
		Extractant					
Phenolic acids							
Gallic acid	3.426	+	+	+	+	+	+
Chlorogenic acid	3.530	+	+	+	+	+	
Caffeic acid	5.077	-	-	-	+	-	-
Chicory acid	6.026	-	-	-	+	-	-
Ferulic acid	7.087	-	-	-	+	+	-
Rosemary acid	12.37	-	-	-	-	-	-
Flavonoids							
Routine	8.394	-	-	-	-	-	-
Luteolin-7-glycoside	13.37	-	-	-	+	-	-
Quercetin	26.6	-	-	-	+	-	+
Dihydroquercetin	30.24	-	-	-	-	-	-
Kaempfero	56.26	+	+	+	+	+	+
Apigenin	62.03	-	-	-	+	-	-
Tannins							
Tannin	3.87	-	-	-	+	-	+
Coumarins							
Umbelliferone	2.82	+	+	+	+	+	+
Coumarin	11.05	-	-	-	-	-	-

Table 12. DPPH radical scavenging activity ethanolic extracts of *Salvia sclarea* L.

Ethanolic extracts		
Concentration. (mg/mL)	Quercetin (mkg/mL)	DPPH scavenging activity (%)
10	250	79.80±0.14
5	125	63.6±0.14
1	62.5	51.7±0.14
0.5	32.25	34.3±0.14

Table 13. Antimicrobial activity of clary sage against various microorganisms

Sample	Microorganism test				
	Bacillus subtilis-5	*Escherichia coli*-221	*Staphylococcus aureus*-91	*Pseudomonas aeruginosa*-225	*Candida albicans*-247
Salvia Sclarea L.	12 мм	-	-	-	12 мм



a) *b)*

Figure 10. Inhibition area of microorganisms *Bacillus subtilis-5* (a) and *Candida albicans-247* on Petri dishes (b)

suggest that n-butanol, particularly in combination with the TERIA-S biofertilizer, is the most effective solvent for extracting a broad spectrum of phenolic compounds from clary sage.

Antioxodant and antimicrobial activity of *Salvia sclarea* L.

The results of the DPPH radical scavenging activity of Salvia sclarea ethanol extracts are displayed in Table 12. Salvia sclarea ethanol extracts were tested at varying strengths and dosages. At low dosages, the radical scavenging effects of various extract percentages did not appear to differ much. Nonetheless, the data showed that the phenolic content and DPPH radical scavenging activity were positively correlated. At 250 µg/ml, the maximum antioxidant capacity was 79.80 ± 0.14%. In a similar experiment by Yasemin Yücel, when studying the antioxidant activity of clary sage in an amount of 240 µg/ml, the antioxidant capacity was slightly higher than in our experiment (Yücel and Nath, 2023). As mentioned above, antioxidant capacity can be affected by various factors such as location, weather conditions, etc.

Based on the obtained data, it was found that ethanol fractions of clary sage exhibited antimicrobial activity against *Bacillus subtilis* (12 mm) and *Candida albicans* (12 mm), but were inactive against the microorganisms *Escherichia coli*-221, *Staphylococcus aureus*-91, *Pseudomonas aeruginosa*-225 (Table 13 and Figure 10).

CONCLUSIONS

The treatment variant incorporating the TERIA-S bioactivator demonstrated superior outcomes in terms of plant growth and biomass accumulation compared to the control across both laboratory and field conditions. In both experimental settings, the application of the bioactivator positively influenced root system development. Notably, under laboratory conditions, plants treated with the bioactivator exhibited accelerated shoot emergence relative to the control; however, shoot

emergence occurred simultaneously in both variants under field conditions.

According to the results of the study, the soil was classified as rich in humus, fairly rich in nitrogen, rich in phosphorus, extremely poor in potassium and poor in microelements (Ca, Mg, Na, K). Microbiological examination of the original soil showed that the amount of ammonifying bacteria, oligonitrophilic microorganisms, micromycetes and actinomycetes was within the normal range. On the other hand, nitrogen-fixing bacteria were present in significantly smaller quantities than expected, and phosphate-mobilizing bacteria were not detected. Depending on the results of the soil analysis, nitrogen-fixing bacteria in the composition of TERIA-S were used to create more favorable conditions for the growth and development of *Salvia sclarea* L.

The application of the environmentally friendly bacterial biofertilizer TERIA-S for presowing seed treatment significantly stimulated the development of both the root system and the aerial parts of Salvia sclarea (clary sage).

HPLC analysis revealed the presence of kaempferol in all clary sage samples. Quercetin was identified in the butanol (experimental) and ethyl acetate (experimental) fractions, while apigenin and luteolin-7-glycoside were detected exclusively in the butanol fraction from the experimental group. Among all extractants, butanol extracted a larger amount of phenolic compounds.

Ethanol fractions of clary sage demonstrated notable antioxidant activity, measured at 79.80 ± 0.14%. Additionally, these fractions exhibited antimicrobial properties, showing inhibition zones of 12 mm against both *Bacillus subtilis* and *Candida albicans*. However, the antimicrobial activity of the substances obtained from Clary sage did not affect Escherichia *coli-221, Staphylococcus aureus*-91, *Pseudomonas aeruginosa*-225.

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